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POTATO EXTRACTION

Determination of End Point in Extraction of Free Amino Acids from Potatoes

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A satisfactory batchwise procedure for the extraction of the free amino acids from potatoes has been developed. Lysine and arginine are the last of the amino acids to be extracted by 70% aqueous ethyl alcohol using this procedure. The order of extraction of the amino acids in potatoes was very different from that reported in young corn shoots. Methods used for the extraction of plant constituents should be tested on each plant material for which they are to be used.

AQUEOUS ETHYL ALCOHOL, 70 to 80%, has been recommended and used (*1–6*, *10*, *13*) to extract the free amino acids from plant materials; however, the workers were not very specific as to when the extraction was complete. Woodward and Rabideau (*17*) have reported on the completeness of extraction of amino acids and other components in corn. They used hot 80% ethyl alcohol in a Soxhlet apparatus, which is known to cause destruction of glutamine (*14*), and as the authors wished to obtain this compound essentially unchanged, their procedure was unsatisfactory.

In the case of sugars, Williams and Potter (*15*) found, "that the sugar solution entrapped in the spongy plant marial is of the same concentration as the remainder of the solution" and "that the alcohol-insoluble material does not occupy a significant volume." This procedure did not work in extracting amino acids from potatoes.

This study agrees with the Woodward and Rabideau (*17*) and Oland and Yemm (*9*) findings that the amino acids are extracted at different rates. Hence, the extraction must be complete or the relative amounts of the different amino acids in an extract will depend on the extent of extraction. Woodward and Rabideau found that the last amino acids to be removed from young corn shoots were aspartic and glutamic acids. Oland and Yemm found arginine to be the last amino acid to be extracted from apple twigs. Arginine and lysine—with a slight emphasis on the latter—were the last amino acids to be removed from potatoes, according to the present work. Methods used for the extraction of constituents from materials derived from different plant species should be tested on each plant material for which they are used.

Experimental

The equilibrium procedure of Williams and Potter (*15*) was compared with a batchwise procedure using Wisconsin Russet potatoes of specific gravity 1.076, which corresponds to a solids content of approximately 19%. The potatoes were hand-peeled and slurried in ethyl alcohol in an electric blender at high speed (10,000 r.p.m.) for 3 minutes. The slurry was transferred to a sampling blender with sufficient alcohol to make the final concentration 70% by weight, taking into consideration the water originally in the potatoes. Samples were taken for the extraction procedures and for nitrogen analyses with the blender running at low speed. The actual amount taken was determined by weight.

Table I. Progressive Changes in the Nitrogen Content of Potato Extracts during Extraction

	Wisconsin Russets			Maine Russets
	Mg. Kjeldahl N / G. fresh wt.	% of total N	μMole leucine equiv.[a] / G. fresh wt.	μMole leucine equiv.[a] / G. fresh wt.
Slurry	2.917	100	...	...
Extracts				
1	1.226	42.0	45.19	52.13
2	0.245	8.40	8.51	12.65
3	0.0867	2.97	2.50	2.65
4	0.0318	1.09	0.724	0.596
5	0.0184	0.63	0.328	0.344
(Soxhlet)				
6	0.0407	1.40	0.645	0.307
7	0.0181	0.62	0.307	0.380
8	0.0086	0.30	0.098	lost
9	...	...	0.061	0.173

[a] Ninhydrin-reactive material as μmoles of leucine equivalents per gram fresh weight (*8*).

Table II. Progress of Extraction of Lysine and Arginine from Wisconsin Russet Potatoes

Extract No.[b]	Lysine N in Extract / Total N in Potatoes × 100	Lysine[a] per Extract / Total Extractable Lysine × 100	Arginine N in Extract / Total N in Potatoes × 100	Arginine[a] per Extract / Total Extractable Arginine × 100
1	0.48	25	2.04	32
3	0.17	8.7	0.54	8.5
5	0.10	5.2	0.25	3.9
(Soxhlet)				
6	0.24	12.5	0.44	6.9
8	0.04	2.1	0.07	1.1
9	0.00	0.0	0.00	0.0
Totals	1.92 (max.) 1.34 (min.)		6.36 (max.) 4.20 (min.)	

[a] Minimum values, using maximum value as total (see text).
[b] Extracts 2, 4, and 7 not analyzed by Moore-Stein technique in interest of time.

Table III. Amino Acid Content of Maine Russet Potatoes

Amino Acid	First Extract, μMole / G. fresh wt.	Combined Extracts, μMole / G. fresh wt.
1	0.18[a]	0.26[a]
2	0.04[a]	0.07[a]
2a	...	0.03[a]
3	0.07[a]	0.02[a]
4	0.05[a]	0.05[a]
5 Aspartic acid	3.17	5.32
6 Threonine	1.59	1.51
7 Serine, glutamine, asparagine[b]	21.78	31.85
8 Glutamic acid	3.78	5.66
9 Proline	0.76	0.90
10 Glycine	0.23	0.33
11 Alanine	0.50	0.55
12 Cystine	0.05	0.09
13 Valine	2.66	2.74
14	0.03[a]	0.04[a]
15 Methionine	0.35	0.43
16 Isoleucine	0.70	0.77
17 Leucine	0.46	0.44
18 Tyrosine	1.03	1.01
19 Phenylalanine	0.93	1.14
20 β-Alanine Tyrosine + phenylalanine + β-alanine	0.14 2.10	0.16 2.44
21 γ-Aminobutyric acid	3.50	3.67
22 Tryptophan	0.25	0.17
23 Histidine	0.50	0.68
24 Lysine	0.58	1.32
25 Ammonia	5.93	4.17
26 Arginine	1.03	2.88

[a] Calculated as micromoles of leucine equivalents as their identity has not been determined. [b] Calculated as asparagine.

Each sample for the extraction tests contained about 100 grams of fresh potato. The samples were made to volume with 70% aqueous ethyl alcohol by weight and mixed thoroughly by shaking. The equilibrium samples were made up to 2, 4, and 8 liters and allowed to stand with occasional shaking. Equivalent aliquots of the supernatant liquid were removed after 2 hours and 1, 2, and 5 days. The aliquots were filtered to remove small amounts of suspended material.

The batchwise sample was made up to about 800 to 900 ml. and swirled more or less continuously for 15 minutes. The mixture was filtered by suction on paper, and the cake was rinsed with 70% alcohol to give combined filtrate and washings of approximately 1 liter. The filter cake was immediately redispersed in 70% alcohol by shaking for 15 minutes. (Preliminary experiments indicated that dispersal in a high-speed blender might cause breakdown of higher molecular weight materials to the point where they would be partly extracted.) The first extract was made up to a 1-liter volume before aliquots were removed for analysis. The later extracts were concentrated under reduced pressure at a temperature below 40° C. and then made up to convenient volumes.

After five extractions were carried out as above, the cake was transferred to a Soxhlet extraction thimble and extracted in the Soxhlet apparatus using about 300 ml. of 70% ethyl alcohol. The apparatus siphoned about four times per hour. The alcohol was changed at the end of each 8-hour period. Four Soxhlet extracts were obtained. The batchwise procedure was repeated later using some sprouted Maine Russet potatoes reported to contain 20.2% solids.

Total nitrogen in the slurry and in the various extracts was estimated by Kjeldahl nitrogen determinations (*16*). Ninhydrin-reactive material in the extracts was estimated by a photometric procedure (*8*). The amino acid composition of the extracts was checked by the Moore and Stein ion exchange procedure (*7*) as modified in this laboratory (*11*).

Results and Discussions

In the equilibrium procedure, as the volume of alcohol used per 100 grams of comminuted fresh potato was increased, the amount of nitrogen extracted approached, but did not reach, a limiting value even when 8 liters of alcohol were used. Only 53.1% of the original nitrogen [estimated by Kjeldahl method (*16*)] was extracted as compared with 57.4% obtained with the batchwise method described above. The figure for 4 liters was 49.5% and for 2 liters was 47.2%. This discrepancy was not due to a time factor as aliquots separated from the potato slurry in less than 2 hours gave the same results as later aliquots that had remained in contact with the potato solids for periods up to 5 days.

Table I compares the nitrogen contents of the two batchwise extracts obtained. These results, however, show only part of the story, because the composition of the individual extracts changes during extraction. Data obtained from the Wisconsin Russets (Table II) show how these changes occur during the extraction of lysine and arginine, the last two amino acids to be removed. Even though not all the extracts were analyzed, one can set maximum and minimum limits for the total extractable compound by assuming that those extracts not reported would contain not more than the immediately preceding extract or less

than the immediately following extract. Although the ninth extract did show ninhydrin activity when tested directly against the ninhydrin reagent (*8*), ion exchange chromatography experiments, using the Moore and Stein procedure (*7*), showed no definite peaks in the effluent curve.

The fourth and fifth extracts were estimated to contain very few acid and neutral amino acids, these having been essentially removed in the first three extracts. Only four peaks caused by this group were found in the fourth extract. The largest of these, the asparagine peak (which may contain glutamine and possibly serine) accounted for only 0.43% of the original nitrogen in the potatoes or about 0.75% of the extracted nitrogen. (As seen from Table III, this peak accounts for most of the extractable nitrogen.) In terms of leucine equivalents, the other three peaks amounted to only 22% of the asparagine peak. In the fifth extract, the asparagine peak amounted to only 0.006% of the original nitrogen and only two other smaller peaks were found in this group. The sixth (first Soxhlet) extract contained only slightly more of the acid and neutral amino acids than did the fifth. Thus essentially all of the acid and neutral amino acids are removed when 40 to 50% of the lysine and 30 to 40% of the arginine are still unextracted.

Fortunately, the acid and neutral amino acids are easily removed, as one of these, glutamine, would be converted by heat into pyroglutamic acid (*14*) if it were not essentially removed before Soxhlet extraction is begun. While asparagine is not completely stable to heat (*12*), small amounts of it remaining until the Soxhlet extraction would not significantly change the results. Other members of the acid and neutral fractions are probably more stable than asparagine.

The results of the second batch extraction (Table I, column 5 closely duplicated the first except that the Soxhlet extraction was not quite as efficient as before. Table III gives the amino acid estimations (*7*, *8*) for the first extract and for the combined aliquots of all the extracts except number eight, which was lost. Some of the other individual extracts were also checked, though the results are not included in the table. Expressed as percentage of amino acids found in the combined extracts, the seventh and ninth extracts contained 9.5 and 3.8%, respectively, of the lysine, and 5.4 and 2.0% of the arginine, while the fourth and fifth extracts contained 0.14 and 0.10% of the acid and neutral amino acids. In Table III, the relative amounts of the amino acids do not differ greatly in the two columns except in the case of lysine and arginine. In a few cases—i.e., threonine—the amount estimated in the combined aliquots was less than in the first extract—probably because of poor separation between peaks and the resultant error in deciding the correct amount to allot to each peak.

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